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THE RELATIVE ACTIVITY OF THE SOLUBLE LIPASE AND LIPASE LIBERATED DURING AUTOLYSIS OF CERTAIN RAPIDLY GROWING TUBERCLE BACILLI

STUDIES IN ACID-FAST BACTERIA. VII *

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In the previous article,[1] experiments were recorded which demonstrated that an active fat-splitting ferment was present in solution in broth cultures of a variety of acid-fast bacilli. These experiments were carried out in such a manner as to be roughly quantitative, and the activity of the lipase, as determined by these experiments, was found to be very considerable. It is a matter of importance to determine whether these lipases are excreted by these acid-fast bacilli as exo-ferments, or whether they are bound up in the bodies of the bacteria, and appear in solution only when the bacteria have autolyzed (endo-ferments). Carrière[2] has described a thermolabile lipase in the organisms of a six-months-old culture of the tubercle bacillus; his observations suggest that this lipase was an endo-ferment and that even minute amounts of culture medium destroy or inactivate it. This observation is not in harmony with the results presented above, for the lipase of the acid-fast bacteria described there was present and active in the culture medium, and it was thermostabile. It is possible, however, that the lipase, which is demonstrable in younger cultures of tubercle bacilli, may have disappeared, as the cultures were incubated for a longer period, without affecting the activity of a second thermolabile endo-lipase which is locked up within the bacteria themselves.

The following observations were undertaken with a view of determining the relative amounts of lipase, respectively, in the culture medium and in the bodies of the bacteria grown in the culture medium. The cultures (various acid-fast bacilli) were of unequal ages, as indicated in Table 1. Various degrees of autolysis of the bacteria, therefore, must have taken place, depending on the strain of organism studied and the length of incubation.

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[1] Study VI.

[2] Compt. rend. Soc. de biol., 1901, 53, p. 320.

The plan of procedure was as follows: For the determination of the lipase in solution in culture media, the method described in the previous article[3] was followed exactly. In order to determine the activity of the lipase present in the organisms themselves (which form a tenacious pellicle on the surface of the media), the bacteria were first separated quantitatively from the culture medium and washed free of adherent soluble lipase by filtration through filter paper, then washed three successive times with redistilled water, each time with 100 c.c.

The organisms, freed from all adherent culture medium by this thorough washing, were suspended in 10 c.c. of redistilled, freshly boiled water containing toluene to kill them, then they were mixed intimately with 1 c.c. of ethyl butyrate and 1 c.c. of castor oil, respectively; and again shaken after the further addition of 0.5 c.c. of toluene. A second set of experiments, using parallel cultures of the same bacteria was prepared, using castor oil in place of ethyl butyrate as the substrate. The filtrates of these parallel cultures gave practically identical results, and, for the sake of brevity, but one set of results is reported under "filtrates," as shown in Table 1. The suspensions containing the bacteria were then neutralized to phenolphthalein, using N/50 acid or alkali, as the case demanded. It should be remarked parenthetically that, without exception, the reaction of the organisms after this washing was practically neutral, so that one or, at most, two drops of N/50 acid or alkali were required to bring the reaction to the neutral point of phenolphthalein. The bacteria and the filtrates, prepared according to the procedure outlined, with suitable media and ester controls, were then incubated at 37 C. for twenty-four hours, and the increase in acidity determined by titration with N/50 NaOH. The suspensions of bacteria were further titrated for three successive days, at the end of which time the activity of the lipase was greatly diminished.

TABLE 1

AMOUNT OF LIPASE IN MEDIUM AND IN BODIES OF BACTERIA

Organisms	Medium	Age	Cc. N/50 NaOH			
			Filtrate		Bacteria	
			Ethyl Butyrate	Castor Oil	Ethyl Butyrate	Castor Oil
B. tuberculosis W	Glycerin	3 months	1.25	1.00	4.05	3.80
B. tuberculosis 597	Mannite	2 months	1.10	0.60	2.30	2.10
B. tuberculosis-Human X	Glycerin	3 months	1.35	1.20	3.30	2.25
B. tuberculosis-Human	Glycerin	3 months	1.00	0.90	2.30	1.70
B. tuberculosis-Human 257	Glycerin	1 month	1.30	0.95	3.35	2.55
B. tuberculosis-Avian	Glycerin	1 month	1.60	1.40	3.70	2.90
B. tuberculosis-Bovine	Glycerin	3 months	1.95	1.40	3.55	2.45
B. leprae (Duval)	Glycerin	3 weeks	0.65	—	4.10	—
B. grass III	Glycerin	3 weeks	1.60	—	5.60	—
B. smegmatis	Glycerin	3 weeks	1.55	—	3.55	—
Control	Glycerin	—	0.00	0.00	0.00	0.00
Control	—	—	0.00	0.00.	0.00	0.00

[3] Study VI.

Table 1 shows the lipase content, respectively, of the broth and the bacteria in terms of cubic centimeters of N/50 alkali. It will be observed that the determination of lipase in the broth was not carried beyond the twenty-fourth hour of incubation; with the bacteria, however, daily titrations were made, the observations being prolonged for three days. The amount of lipase in the filtrate was found to be between 0.65 and 2.00 c.c. of N/50 acid, but it must be remembered that the total lipase content of the 100 c.c. of the broth, from which the bacteria were derived, would be one hundred times this amount, theoretically. The amount of lipolytic activity exhibited by these organisms, while considerable in amount, is noticeably less than the total theoretical fat-splitting power of the underlying culture medium. The cultures had been incubated for the most part from one to three months, so that it would be justifiable to assume considerable autolysis before these determinations were made. Nevertheless, the very considerable disparity observed between the relatively large amount of lipolytic activity of the filtrates on the one hand, and of the bacteria which grew in these culture media on the other, would suggest the possibility that the ferment in the culture medium, in part at least, was an extracellular lipase, unless it be assumed that this lipase was an endo-ferment which had escaped from the substance of the bacteria into the underlying medium during their autolysis prior to these experiments. The experiment does not determine this point.

TABLE 2

AMOUNT OF LIPASE IN WASHINGS AND IN BACTERIA AUTOLYZED AFTER WASHING

Organisms	Medium	Age	Cc. N/50 NaOH to Neutralize Ethyl Butyrate							
			24 Hours Incubation				Bacteria Autolyzed after Washing			
			Filtrate from Bacteria	Washings			Incubation			
				A	B	C	24 Hrs.	48 Hrs.	72 Hrs.	Total
B. tuberculosis W	Plain broth	6 weeks	0.65	0.05	0.00	0.00	2.35	2.30	0.30	4.95
B. tuberculosis W	Dextrose broth	6 weeks	1.30	0.10	0.00	0.00	2.80	2.90	0.75	6.45
B. tuberculosis W	Mannite broth	6 weeks	1.15	0.05	0.00	0.00	2.35	2.20	0.20	4.75
B. tuberculosis W	Glycerin broth	6 weeks	1.75	0.05	0.00	0.00	2.30	1.60	0.30	4.20
B. tuberculosis 597	Plain broth	6 weeks	0.45	0.05	0.00	0.00	2.20	2.15	0.50	4.85
B. tuberculosis 597	Dextrose broth	6 weeks	1.20	0.10	0.00	0.00	2.85	3.30	0.15	6.30
B. tuberculosis 597	Glycerin broth	6 weeks	1.70	0.20	0.00	0.00	3.15	2.25	0.60	6.00
Control	Medium and ester	—	0.00	....	....	....	....	....	....	....
Control	Wash'gs and ester	......	....	0.00	0.00	0.00	....	....	....	....
Control	Ester	......	0.00	....	....	....	0.00	....	....	....

In order to determine the amount of lipase lost in washing these bacteria and to throw some light on the possible influence of the source of carbon on the production of lipase, an experiment was made as follows: two rapidly growing human tubercle bacilli, "W" and "597," were grown for six weeks in plain, dextrose, mannite, and glycerin broths, respectively. At the end of that time the organisms were separated quantitatively from the underlying medium by filtration through filter paper, then they were washed with three successive portions of distilled water. The washings were labelled A, B, and C, respectively. The determinations were made as indicated in Table 2.

The results indicate that the filtrates from plain broth cultures were uniformly less active lipolytically than the filtrates of the same organisms grown in dextrose, mannite, and glycerin broths. There was very little lipase in the first washing (A), none in the second and third washings (B and C). The results otherwise are self-explanatory.

CONCLUSIONS

The observations appear to justify the conclusion that certain acid-fast bacteria grown in nutrient broth, with dextrose, mannite, and glycerin as additional sources of carbon, produce an active lipase which appears in solution in the various culture media.

The bodies of the bacteria, freed from adherent culture media and soluble lipase by thorough washing, also contain an active lipase, probably liberated as the bacteria underwent autolysis.

The lipase in solution appears to be either greater in amount, or more active than that contained in the bacteria freed from the culture medium.

It cannot be stated whether the lipase free in the culture media is freed as the result of autolysis of the bacterial cells (endo-lipase), or whether it is secreted by the bacteria as an exo-lipase.